

20100207 11:20:00

# Western Blot of Flk-1/SEAPS Immunoprecipitation with MAb DC101

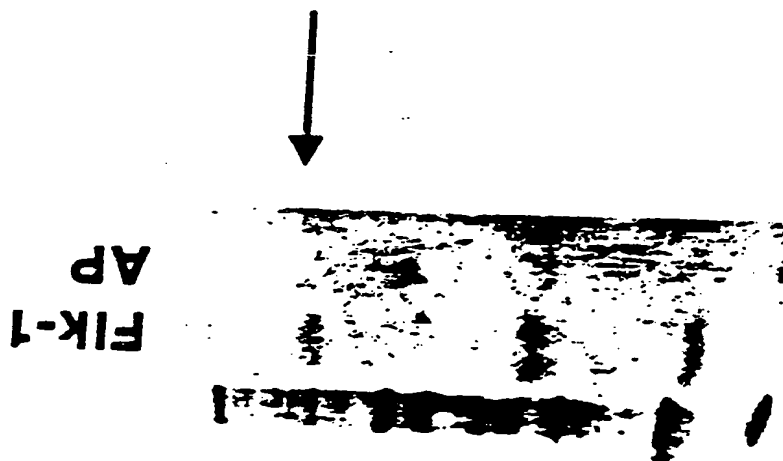
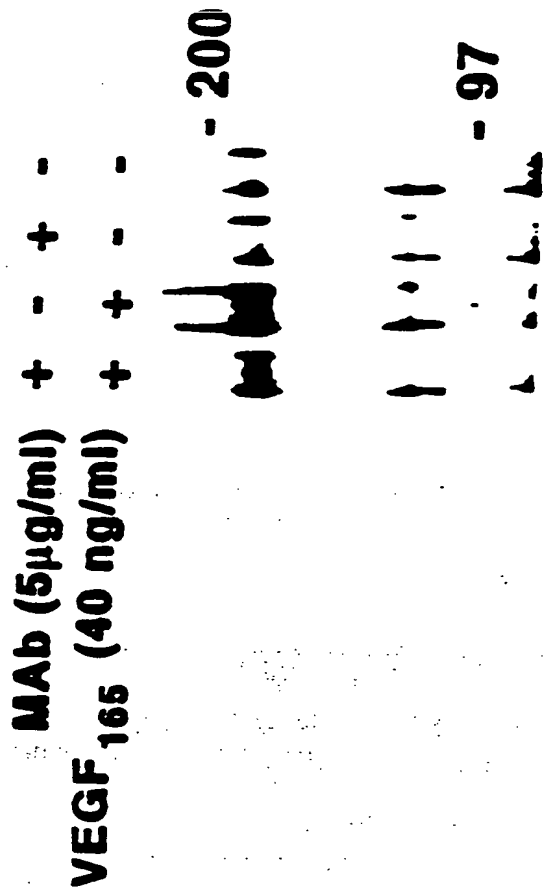


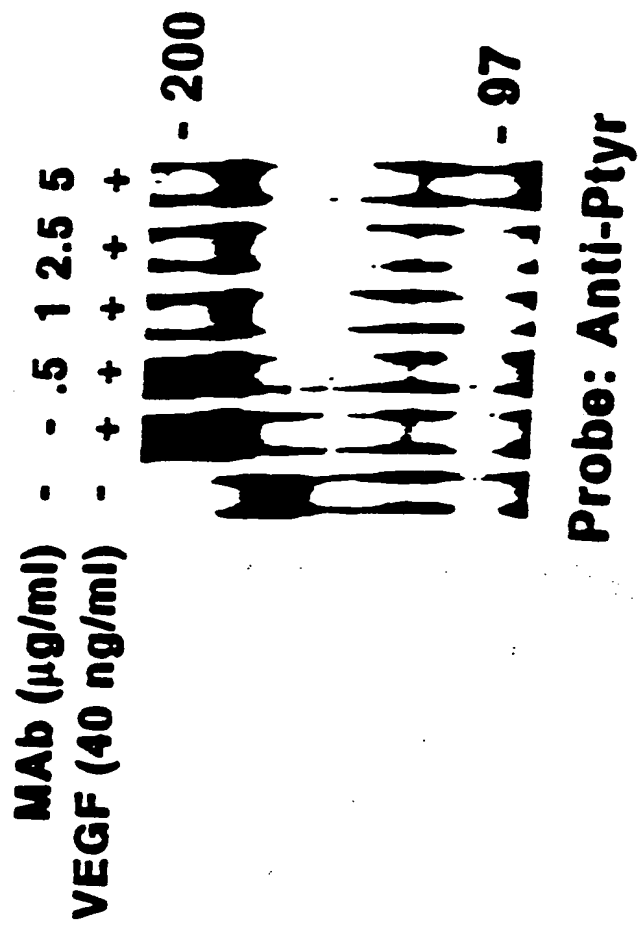
Figure 1

**Figure 2a**



**Probe: Anti-Ptyr**

### Figure 2b



MAB (5µg/ml) + + + + +  
 VEGF (ng/ml) 80 40 20 10 0 -  
 - 200

Anti-pTyr

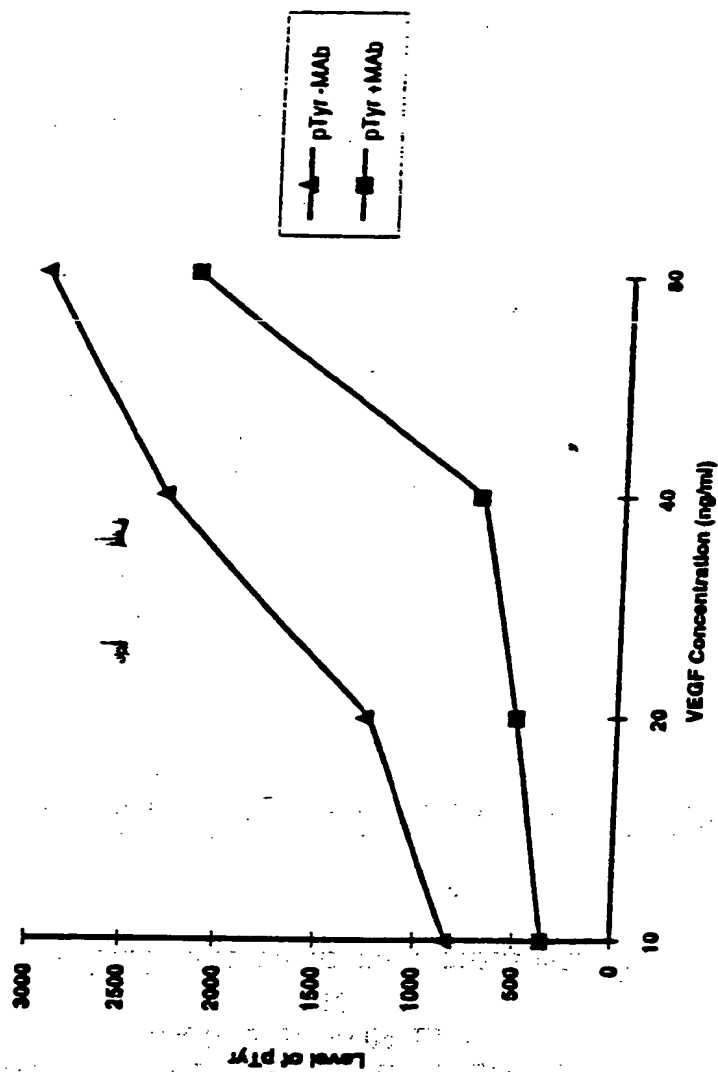
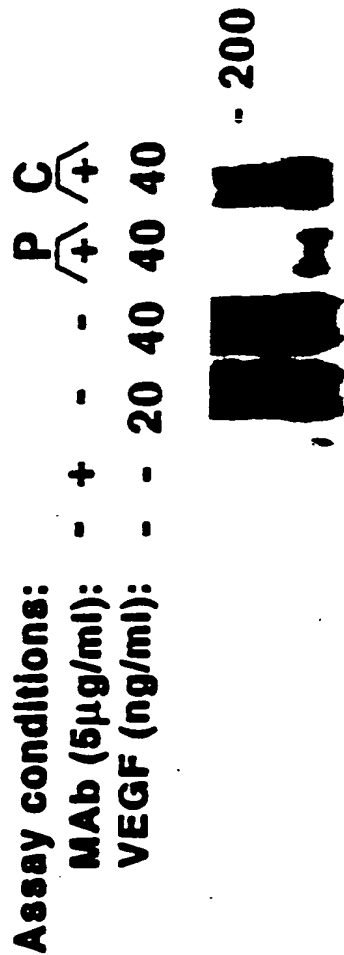


Figure 3

# **Inhibition of VEGF-Flk-1/fms activation by prebound MAb DC101**

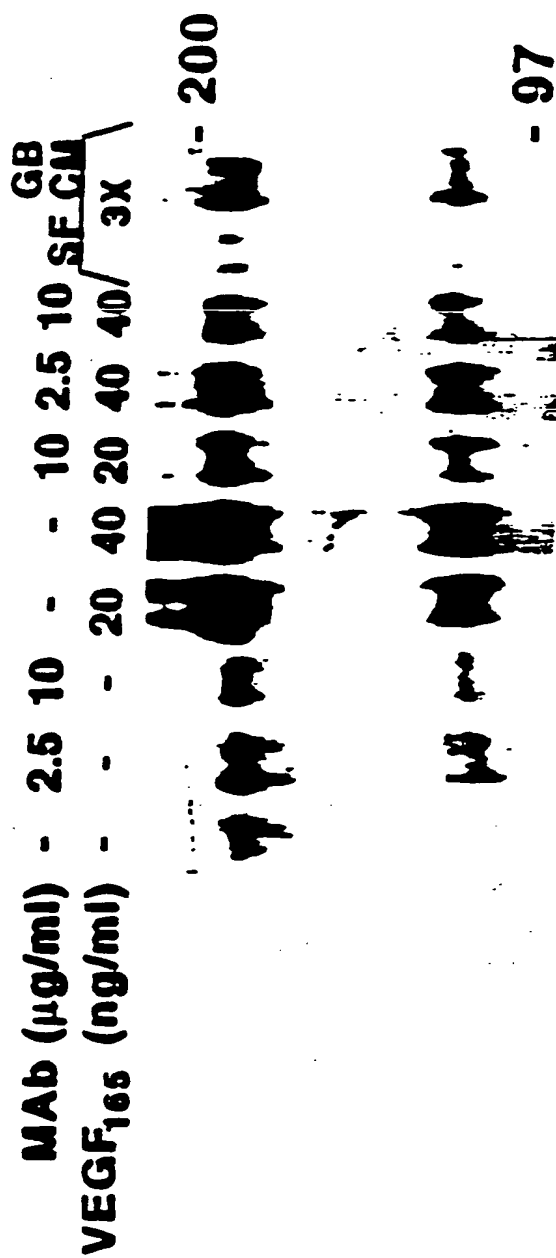


**Probe: Anti-Ptyr**

**Assay conditions:** P: MAb prebound 15'; VEGF 15'  
 C: Competitive assay; MAb + VEGF 15'

**Figure 4**

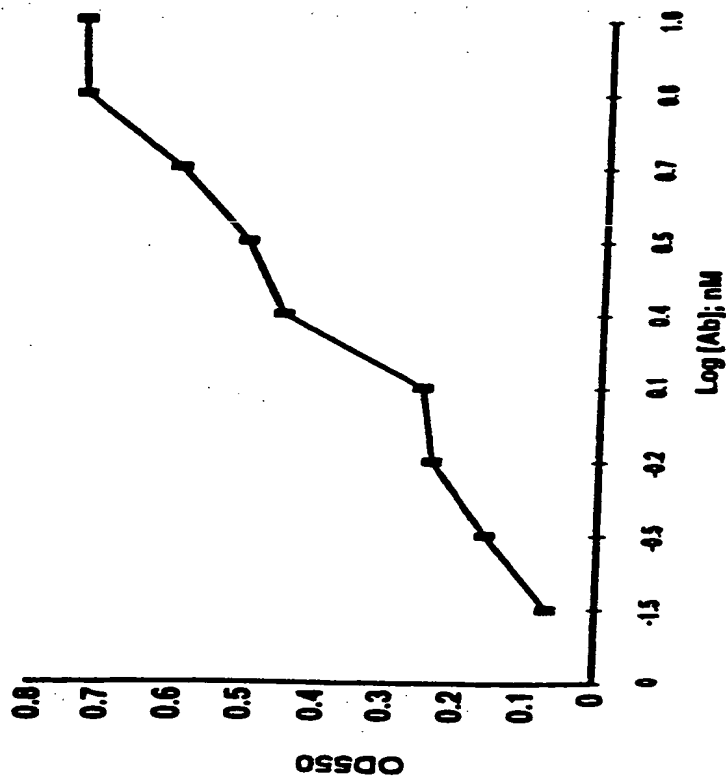
**Figure 5**



**Probe: Anti-Ptyr**



# **Saturation Binding of MAb DC101 to the Flk-1/fms Receptor on the Transfected 3T3 Cell Line C441**



**Figure 7**



# **Immunoprecipitation of phosphorylated flk-1/fms from VEGF stimulated flk-1/fms transfected 3T3 cells.**

**Antibody: 1 2 3 4**

**■ ■ - 200**

**Anti-pTyr**

- Antibodies:**
- 1) Rat anti-flk-2 IgG<sub>2a</sub> 2A13**
  - 2) Rat anti-flk-1 IgG<sub>1</sub> DC101**
  - 3) Rat anti-flk-2 IgG<sub>1</sub> 23H7**
  - 4) Rabbit anti-fms polyclonal IM 133**

**Figure 8**

# S nsitivity of VEGF induced phosphorylation of the Flk-1/fms receptor to inhibition by MAb DC101

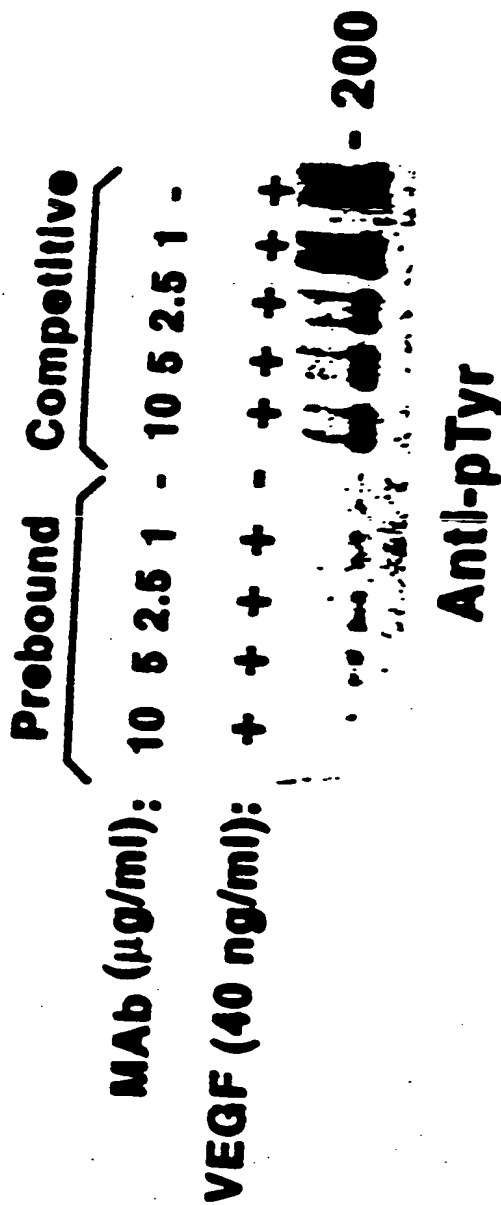
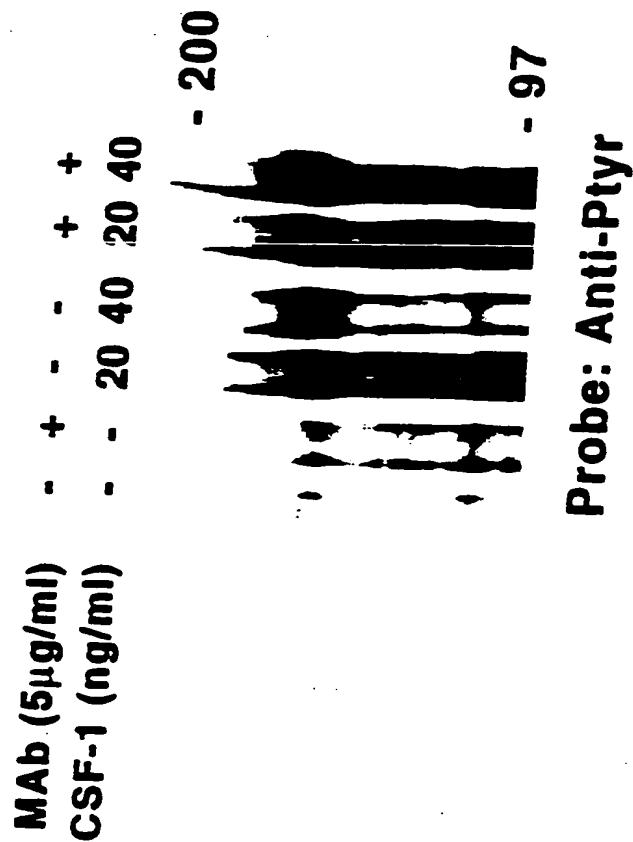




Figure 9

# **Effect of MAb DC101 on CSF-1 induced phosphorylation of the FMS receptor.**



**Figure 10**

# Specificity of MAb DC101 neutralization of the activated flk-1/fms receptor

Condition:	Competitive	Prebound
VEGF ng/ml:	- - - 20 40 40 40	40 40 40
MAb:	1 2 3 - - 1 2 3	1 2 3
(5µg/ml)		
	... 	
		- 200

Anti pTyr



Anti-FMS

- Rat MAbs: 1) Anti-flk-1 IgG, DC101  
2) Anti-flk-2 IgG, 23H7  
3) Anti-flk-2 IgG<sub>2a</sub>, 2A13

Figure 11

204050" FT 20600T

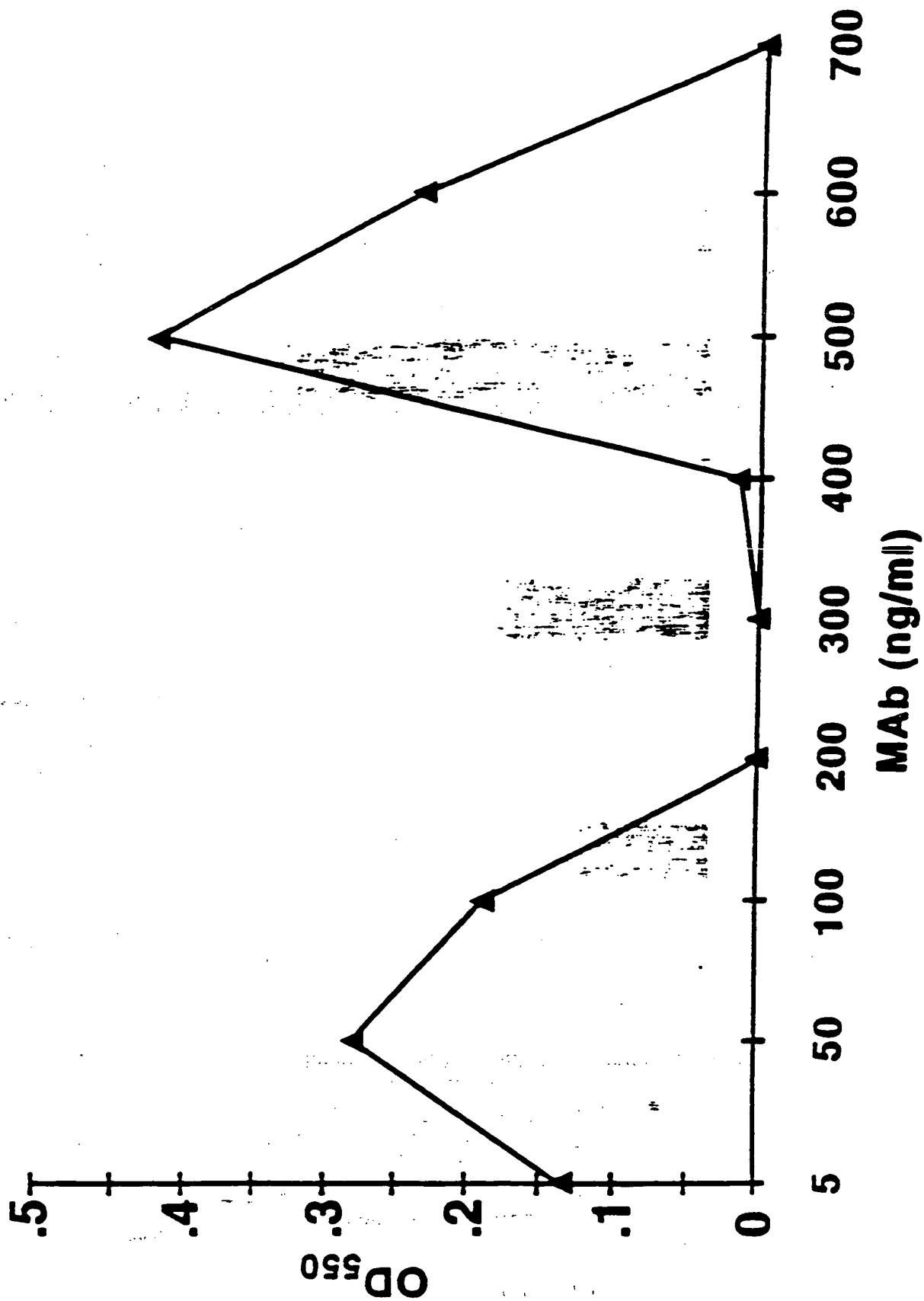


Figure 12

# Immunoprecipitation of phosphorylated receptor bands from VEGF stimulated HUVEC cells

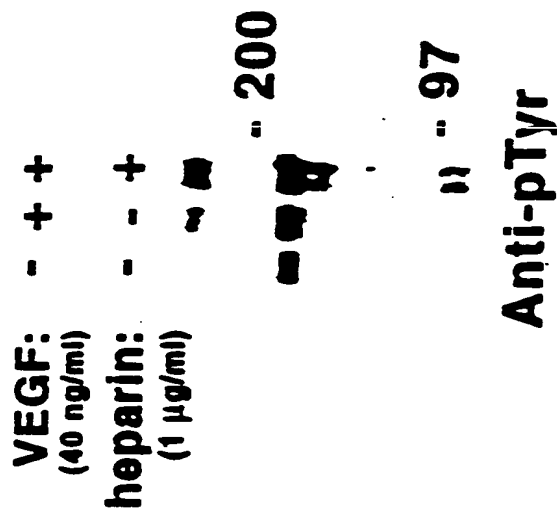


Figure 13

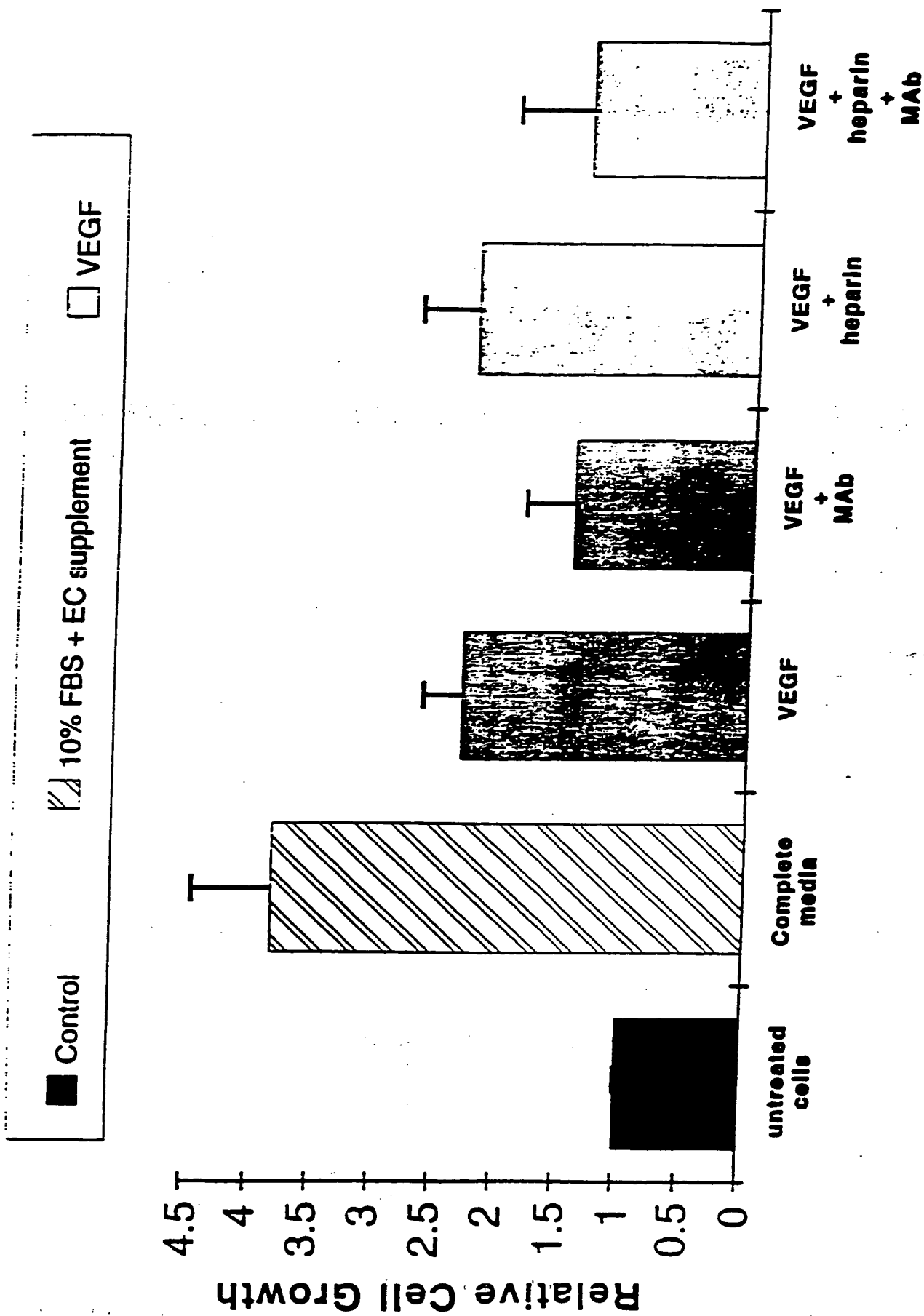
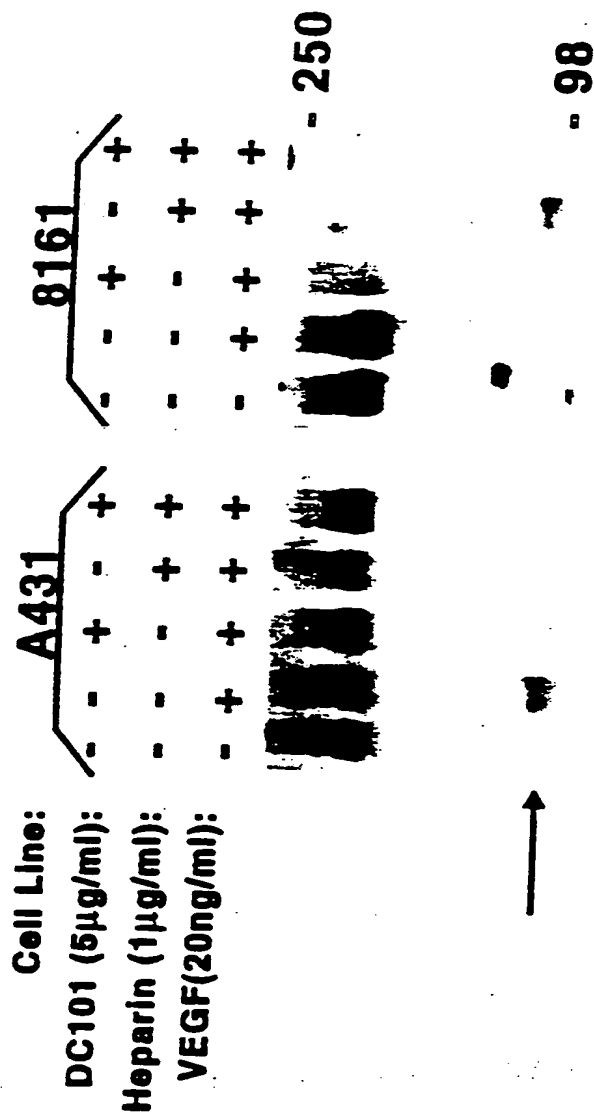


Figure 14

# Effect of MAb DC101 on VEGF Receptor Forms in the Tumor Cell Lines A431 and 8161



IP: MAb DC101

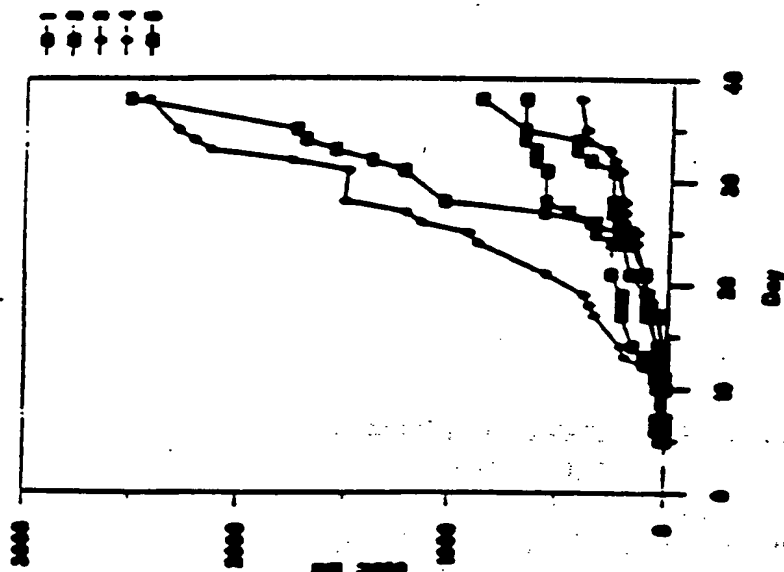
Probe: Anti-pTyr

Figure 15

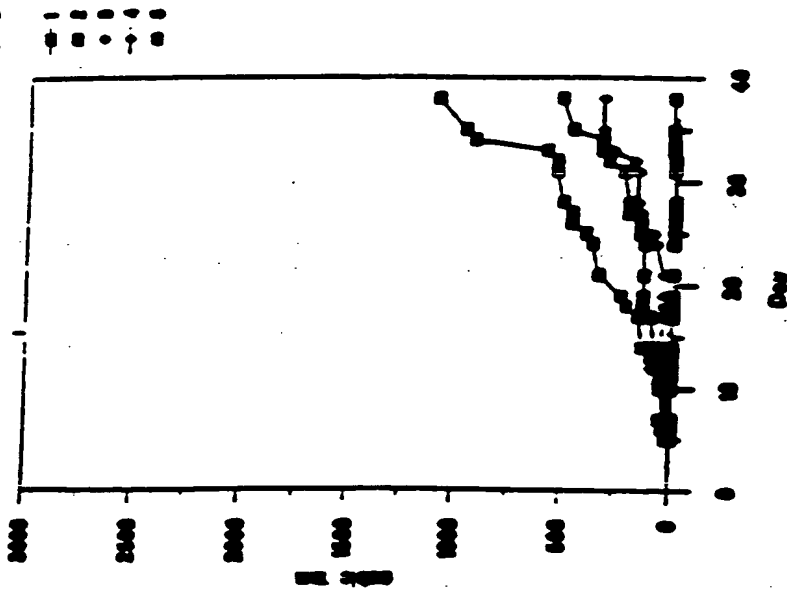


Figure 16  
204060 TT20500T

Treatment of Glioblastoma  
Xenografts with Rat anti-flk-2 MAb



Treatment of Glioblastoma  
Xenografts with Rat anti-Flk-1 MAb



Statistical Analysis:

Flk-1 slope = 16.09

Flk-2 slope = 37.39

p value for Flk-1 versus Flk-2 tumor size = 0.0001

204030 TT 20500T  
**Figure 17**

